Bacterial Infection as a Cause of Cancer

Julie Parsonnet

Departments of Medicine and Health Research and Policy, Stanford University School of Medicine, Stanford, California

Bacterial infections traditionally have not been considered major causes of cancer. Recently, however, bacteria have been linked to cancer by two mechanisms: induction of chronic inflammation and production of carcinogenic bacterial metabolites. The most specific example of the inflammatory mechanism of carcinogenesis is *Helicobacter pylori* infection. *H. pylori* has been epidemiologically linked to adenocarcinoma of the distal stomach by its propensity to cause lifelong inflammation. This inflammation is in turn thought to cause cancer by inducing cell proliferation and production of mutagenic free radicals and *N*-nitroso compounds. *H. pylori* is the first bacterium to be termed a definite cause of cancer in humans by the International Agency for Research on Cancer. Mutagenic bacterial metabolites are also suspected to increase risk for cancer. This model is best exemplified in colon cancer. Bile salt metabolites increase colonic cell proliferation. Exogenous compounds such as rutin may be metabolized into mutagens by resident colonic flora. Moreover, *Bacteroides* species can produce fecapentaenes, potent *in vitro* mutagens, in relatively high concentrations. *In vivo* data on human carcinogenesis by bacterial metabolites, however, are inconsistent. Local bacterial infections may also predispose to nonnodal lymphomas, although the mechanisms for this are unknown. Gastric lymphomas and immunoproliferative small intestinal disease have been most strongly linked to underlying bacterial infection. Because bacterial infections can be cured with antibiotics, identification of bacterial causes of malignancy could have important implications for cancer prevention. — Environ Health Perspect 103(Suppl 8):263–268 (1995)

Key words: bacteria, neoplasm, gastric cancer, colorectal cancer, *Helicobacter pylori*, epidemiology, carcinogenesis, normal flora

Introduction

A substantial number of bacterial pathogens have been putatively linked to cancer. As early as 1772, *Mycobacterium tuberculosis* was thought to cause malignancy (*1*). It was observed that bronchogenic carcinomas frequently appeared in areas of pulmonary scarring, presumably from tuberculosis. Persons with lung cancer also were noted to have active tuberculosis more frequently than the general population. Like many hypotheses attributing cancer to specific infectious agents, however, the tuberculosis–cancer theory did not stand the test of time. Most bronchogenic carcinomas in persons with tuberculosis do not occur at scar sites but elsewhere in the lung. Furthermore, observed scars at tumor sites now appear to be the result of the malignancy rather than the cause. Any link between active tuberculosis and malignancy is currently ascribed to reactivation of infection in immunocompromised cancer patients rather than to a cause-and-effect relationship between infection and neoplasm (*2–4*).

Despite this early misstep, bacterial theories for carcinogenesis continue to be promulgated (*5*). Now, however, rather than directly attributing cancer to specific organisms, attention has focused on nonspecific mechanisms of carcinogenesis. Two such mechanisms are induction of inflammation, and production of mutagenic compounds by bacterial metabolism. The first mechanism is best exemplified by *Helicobacter pylori* infection and gastric cancer. Colon cancer provides a model for the second of these mechanisms. Yet a third mechanism, that for lymphomas, has yet to be credibly modeled.

Bacteria, Inflammation, and Cancer: the *Helicobacter pylori* Model

Infections have been nonspecifically tied to malignancy through their ability to cause chronic inflammation. Among the chronic inflammatory processes linked to cancer are parasitic infections [e.g., *Schistosoma haematobium* and *Opisthorchis viverrini* (*6,7*)] and viruses [hepatitis B (*8*)]. In general, these infections cause cancer in direct proportion to their chronicity; the longer the inflammatory process persists, the more likely malignancy is to develop (*7,8*). Among bacterial inflammatory processes, chronic osteomyelitis was the first to be convincingly associated with cancer in humans. Constant irritation of a draining sinus tract by inflammatory exudates of the underlying bone predisposes the host to carcinoma of the skin, regardless of the specific bacterial pathogen involved (*9*). Fortunately, in the era of antibiotics chronic osteomyelitis contributes a vanishingly small number of cases to the cancer registry. A similar mechanism has been proposed for bladder cancer in persons with recurrent or persistent cystitis (*10,11*).

Currently, a popular model for bacterial carcinogenesis is that of *Helicobacter pylori* infection and gastric adenocarcinoma. *H. pylori* is a Gram-negative rod that lives in a neutral pH niche between the mucus layer of the stomach and the gastric epithelium. Although *H. pylori* can be found lining the mucus layer adjacent to ectopic gastric tissue (e.g., in Meckel's diverticula), it is never found remote from gastric epithelium and does not invade tissue; it neither enters epithelial cells nor penetrates the basement membrane. Despite this lack of invasion, *H. pylori* infection is invariably associated with inflammation (*12,13*). Once established, *H. pylori* infection and its associated inflammation are thought to last for decades if not a lifetime (*14*). At least 50% of the world's population harbors the organism (*14*).

Recently, *H. pylori* was declared by the International Agency for Research on Cancer (IARC) to be a Group 1 carcinogen, a definite cause of human cancer (*7*).

This paper was presented at the President's Cancer Panel Conference on Avoidable Causes of Cancer held 7–8 April 1994 in Bethesda, Maryland. Manuscript received 9 March 1995; manuscript accepted 24 March 1995.

This work is supported in part by a Junior Faculty Research Award from the American Cancer Society and by a Clinical Epidemiology Fellowship from Merck and the Society for Epidemiologic Research.

Address correspondence to Dr. Julie Parsonnet, Departments of Medicine and Health Research and Policy, HRP Building, Room 225, Stanford University School of Medicine, Stanford, CA 94305. Telephone: (415) 723-7274. Fax: (415) 725-6951. E-mail: ml.jxp@forsythe.stanford.edu

Abbreviations used: IPSID, immunoproliferative small intestinal disease; MALT, mucosal-associated lymphoid tissue; IARC, International Agency for Research on Cancer.

Support for this decision came principally from pathologic studies of the natural history of gastric adenocarcinoma and epidemiologic studies statistically linking *H. pylori* to malignancy.

Chronic superficial gastritis has long been thought to be a precursor lesion to gastric adenocarcinoma (*15,16*). Even in the absence of more advanced preneoplastic lesions, superficial gastritis increases cancer risk 2-fold (*17*). In approximately 3 to 5% of persons per year, superficial gastritis progresses to chronic atrophic gastritis, a more advanced cancer precursor lesion (*18–20*). With extensive atrophy, cancer risk increases up to 9-fold (*17*). As atrophy worsens, patches of intestinal metaplasia arise and the gastric epithelium transforms to either small or large bowel morphology. Cancer ensues thereafter.

Since *H. pylori* causes the vast majority of superficial gastritis, it can be deduced from the data above that *H. pylori* is a likely risk factor for malignancy and increases cancer risk at least 2-fold. The role of *H. pylori* in the development of chronic atrophic gastritis and intestinal metaplasia is unclear. Atrophic gastritis and intestinal metaplasia are inhospitable to *H. pylori,* and biopsies taken from these areas yield no organisms. In spite of this, *H. pylori* often is identified in nonatrophic locations of the same stomach (*21*). Moreover, in most persons with atrophy or intestinal metaplasia, anti-*H. pylori* IgG is found in serologic assays, suggesting smoldering infection (*22,23*). In support of a causal role, one study has suggested that cytotoxin-producing strains of *H. pylori* are more common in persons with chronic atrophic gastritis than in persons with only superficial gastritis without atrophy (*24*).

An animal system to confirm the *H. pylori* cancer model has yet to be established. Thus, epidemiologic studies provide the strongest evidence for the link between *H. pylori* and cancer. Correlations between *H. pylori* prevalence and gastric cancer incidence reveal both geographic and temporal parallels. In two of the largest ecologic studies, Forman et al. found significant correlations between *H. pylori* seroprevalence and gastric cancer rates among 49 rural Chinese counties and 17 nations worldwide (*25,26*). Other investigators observed that the prevalence of *H. pylori* and/or superficial gastritis has declined over time concomitant with the decrease in gastric cancer incidence (*27–29*). Smaller ecological studies yield less consistent results (*23,30,31*). Similarly, while some case–control studies of *H. pylori* in gastric cancer have shown significant associations between infection and malignancy (*32–35*), others have not (*36–40*).

The most convincing data implicating *H. pylori* as a cause of cancer are four nested case–control studies from Hawaii, California, Great Britain, and Taiwan (*41–44*). In the first three studies (mean follow-up 13, 14, and 6 years, respectively), serologic evidence of *H. pylori* infection increased risk of later developing gastric cancer between 2.8- and 6-fold (*41–43*). The fourth nested case–control study also identified an elevated risk of cancer (odds ratio = 1.6), but the finding was not statistically significant (*44*). This last study was hampered, however, by a small number of cases ($n = 29$) and short follow-up period (mean = 3 years). Overall, the association between *H. pylori* and cancer appeared to be restricted to tumors distal to the gastric cardia (*41,43*).

A combined analysis of three nested case–control studies showed the strongest association between infection and cancer (odds ratio = 8.7) when the interval between serum collection and cancer diagnosis was longer than 15 years (*45*). When serum was drawn more proximate to the time cancer occurred, the relative risk estimate was considerably lower (odds ratio = 2.1). This has been observed independently by other investigators (*46*) and suggests that antibody titers, and perhaps the presence of infection itself, might diminish as preneoplastic lesions progress toward cancer.

The epidemiologic and pathologic associations between *H. pylori* and cancer would have little meaning were infection not a biologically plausible cancer risk factor. *H. pylori* infection, like other infectious causes of chronic inflammation, theoretically fits the role of a promoter in the multistage model of carcinogenesis (*6*). Promoters select for clonal expansion of cells either by causing alteration in increasing proliferation and gene expression or by causing changes in terminal cell differentiation (*47,48*). *H. pylori* infection causes increased cell proliferation (*49,50*). Eradication of *H. pylori* decreases cell proliferation, probably because of decreased inflammation rather than loss of the organism itself (*50–52*). Although *H. pylori*-related hyperproliferation is not yet understood, possible causes are direct damage to mucosal cells by *H. pylori*-related factors (i.e., ammonia), trophic effects of increased gastrin production, or indirect damage to the epithelium by the inflammatory response (*49,53–57*). Cell proliferation in turn increases risk for DNA replication error and predisposes mucosal cells to transformation by dietary or endogenous mutagens (*58,59*).

Other elements of inflammation also can be seen as tumor-promoting processes (*48,58*). Inflammatory cells increase conversion of nitrates to nitrites, enhancing the likelihood of *N*-nitrosamine formation (*6*). This may explain the epidemiologic observation that in some populations high dietary nitrates increase gastric cancer risk (*60*). Moreover, activated macrophages produce nitrite, nitrate, and nitrosating agents (*61*). When macrophages are cultured with appropriate amines, *N*-nitroso compounds are formed. Free radicals produced by the inflammatory response, e.g., O^- and superoxide, alter the structure and function of lipids, proteins, and DNA causing changes in cell metabolism and gene expression. Excess production of reactive oxygen species has been noted in human mucosal tissue infected with *H. pylori* (*62,63*). Thus, without being directly genotoxic, *H. pylori* can contribute to the development of uncontrolled cell growth.

Because *H. pylori* is curable with a short course of antibiotics, it is tantalizing to speculate that treatment of infection or creation of a vaccine could prevent gastric cancer. Unfortunately, studies proving this almost certainly will be difficult or impossible to perform. Very large cohorts would be needed with many years of follow-up. Intermediate markers for cancer risk are easier to evaluate, and the effects of therapy on advanced precursor lesions are currently being studied. Pending these results, a preliminary cost-effectiveness analysis suggests that even if treatment prevents only 20% of infection-related cancers, antibiotic therapy could be a reasonable approach to disease prevention in high risk groups such as Japanese Americans and African Americans (*64*).

Colon Cancer and Bacterial Colonization of the Intestine

For obvious reasons, research into the etiology of sporadic colon cancer has focused on dietary influences (*65*). After decades of study, several trends are evident: fats are consistently risk factors for colon cancer and fiber is consistently protective. Mechanisms for these associations are not yet clear. One widely held theory, however, is that risks attributable to foods are mediated by bacterial actions in the intestine.

The intestine harbors an enormous variety of bacterial flora, with colonization becoming more dense progressing from pylorus to distal colon. In the proximal duodenum, few organisms survive. In the colon, on the other hand, it is estimated that 10^{14} organisms of hundreds of different species (the vast majority anaerobes) vie for space and nutrients. Despite the wide diversity of resident organisms, differences in composition of gut flora within human populations have been difficult to substantiate, even in persons with very different diets (*66*).

Bacteria are thought to have several indirect carcinogenic actions in the gut. First, they deconjugate and reduce bile acids. While most bile acids are absorbed in the small intestine, a small percent will pass into the colon. *In vitro,* it has been shown that bacterial species within the colon can deconjugate the 7α-hydroxyl groups from bile acids to produce cytotoxic 7α-dehydroxylating bile acids (deoxycholate and lithocholate) (*67*). These compounds are reported to promote cell proliferation (*68*) and growth of adenomas (*69*). This in turn enhances carcinogenesis by exogenous or endogenous mutagens. Thus, copious secretion of bile acids following fatty meals would increase risk of colon cancer in persons with high fat diets.

Bacteria are also thought to activate exogenous mutagen precursors. Examples observed *in vitro* and *in vivo* are: hydrolysis of rutin to quercetin (a mutagenic aromatic amine), hydrolysis of cycasin to methylazoxymethanol (*70*), and hydrolysis of glucuronide-conjugated polycyclic hydrocarbons to their unconjugated, mutagenic forms (*71*). Fecapentaenes, potent mutagens synthesized by *Bacteroides* species, are also found in relatively high concentrations in human feces, although their relationship to cancer is unproven (*72–74*). Furthermore, bacteria ferment polysaccharides and glycoproteins to volatile fatty acids. These may increase distal colon cell proliferation by altering membrane structure, although, again, *in vivo* support for this is lacking (*75,76*).

Because of the many species of bacteria in the gut (a considerable portion of which remain unidentified), focusing on any one organism as a cause of cancer is a daunting task. It remains possible, however, that specific bacterial species play more direct roles in colon carcinogenesis. In murine models, *Citrobacter freundii* causes attaching and effacing lesions of the large intestine similar to those caused by enteropathogenic *Escherichia coli* in humans (*77*). Animals infected with *Citrobacter freundii* develop colonic hyperplasia and when exposed to exogenous mutagens, progress more rapidly to malignancy than uninfected animals (*78,79*). In humans, efforts have been made to similarly identify specific organisms that cause proliferation and/or malignancy. For example, several cross-sectional studies indicate that certain *Clostridium* species are more common in colon cancer patients than in other subjects, although a causal relationship remains unproven (*80,81*). In light of the *Citrobacter freundii* model, however, it is conceivable that specific bacteria may induce the optimal proliferative environment for mutagens to induce their damage.

Unlike the *H. pylori* model outlined above, there is little hope that antibacterial strategies will play a role in colon cancer prevention. Normal flora are a fundamental component of the human gastrointestinal tract. Since no specific species of organism has been targeted as the colon cancer culprit, no antimicrobial therapy or vaccine can be explored. Until a specific organism is pinpointed, cancer prevention strategies can only focus on diet as it influences bacterial pathogenesis.

Lymphomas

Circumstantially, bacteria appear to be involved in pathogenesis of two types of lymphomas: gastric lymphomas and immunoproliferative small intestinal disease (IPSID). Although the stomach is not a lymphoid organ, gastric lymphoma is the most common extranodal lymphoma. Approximately 20 to 30% of these tumors arise from mucosal-associated lymphoid tissue (MALT). MALT consists of organized lymphoid follicles in mucosal areas of nonlymphoid organs such as the GI tract and thyroid and salivary glands. Recently, Isaacson showed that MALT can be the nidus for B-cell neoplasms called MALT lymphomas (*82*). These low-grade malignancies are characterized by specific histologic features: nonneoplastic lymphoid follicles, centrocytelike cells, lymphoepithelial lesions, and plasma cell differentiation.

One line of research currently favors *H. pylori* infection as a causal factor in both MALT and non-MALT gastric lymphomas. Several facts favor this hypothesis. First, gastric MALT is extraordinarily common in patients with *H. pylori* infection. One pathologist has suggested that all infected subjects will have MALT if the pathologist diligently looks for it (*83*). *H. pylori* is also common in subjects with MALT lymphomas (*84*). Gastric MALT lymphoma cells proliferate when cultured in the presence of T-cells and *H. pylori* antigens (*85*). In a mouse model of *H. felis,* lymphoid follicle formation was followed by MALT-like tumors after several years of sustained infection (*86*). More remarkable still, gastric MALT and MALT lymphomas remit coincident with cure of *H. pylori* infection, although a nonspecific response to antibiotics cannot be ruled out (*87,88*). The only prospective epidemiologic study done in humans, however, found that *H. pylori* infection increased risk for all gastric lymphomas not just MALT lymphomas (odds ratio = 6.3) (*89*). This suggests that infection is driving the proliferation of MALT cells and the progression of MALT to both MALT and non-MALT lymphomas. Another hypothesis is that MALT lymphoma reflects an aberrant autoimmune response to chronic infection (*82,90*).

The second bacterium-related lymphoma, IPSID, is an unusual malignancy that occurs predominantly in young adults of lower socioeconomic regions of the southern and eastern Mediterranean region. Commonly known as Mediterranean lymphoma, IPSID is a MALT-type lymphoma that is almost invariably associated with excessive production of a heavy chains (*91*). Like gastric MALT lymphoma, IPSID responds to antibiotic therapy. When treated in the early stage, up to 40% of tumors will completely regress with antibiotic treatment (typically tetracycline) (*91–93*). The precise reason for these remissions remains unclear. One widely held theory is that IPSID succeeds unremitting bacterial stimulation of lymphocytes (*93*). In particular, recurrent diarrheal disease beginning in infancy has been implicated as a stimulus for uncontrolled lymphoproliferation. Bacterial overgrowth of the small bowel is evident in some cases, although no specific bacterium appears to be more common than in controls (*93*).

Because nonnodal lymphomas are rare, these diseases are difficult to study. Furthermore, the variable pathologic classifications of lymphomas makes consistency among investigators difficult. Even the distinction between lymphoma and lymphoproliferation often is problematic. Thus, the true nature of the relationship between bacteria and lymphomas remains obscure. Whether the bacterial disease model can be applied to other extranodal or non-MALT lymphomas is unknown.

Conclusions

One of the most intimate relationships of man is that which he has with his own microbial flora. While most exposures in life are transient, the contact we have with these microorganisms is constant and unremitting. This symbiotic relationship is taken for granted or, more commonly still, thought to be beneficial. Even the term normal flora suggests benignity. Yet it is naive to assume that our continuous interaction with microbial flora is immaterial to our long-term health. As new infectious causes of malignancy continue to be uncovered, it is increasingly apparent that dissection of the complex interplay between man and microbial flora is essential to understanding the pathogenesis of many malignancies.

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